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FLASH-INDUCED 515 nm ABSORBANCE CHANGE IN CHLOROPLASTS WITH VARIOUS GRANUM CONTENTS

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SUMMARY

The 515 nm absorbance change was studied in mesophyll and bundle sheath chloroplasts of maize, which contain different amounts of grana. The amplitude of the 515 nm signal (induced by 3 μs flashes repeated at 4 s intervals) has shown a correlation with the granum content of the samples. However, upon addition of *N*-methylphenazonium methosulphate the 515 nm signal became independent of the amount of grana: in agranal thylakoids a large pool of silent Photosystem I was activated and, as a result, the amplitude of the 515 nm signal of agranal chloroplasts increased to the level exhibited by granal chloroplasts.

These data show that the 515 nm absorbance change is not limited to small closed vesicles like grana, but in the presence of suitable electron donors single lamellae of bundle sheath chloroplasts can also be active.

INTRODUCTION

The light-induced absorbance change at 515 nm in chloroplasts and in photosynthetic bacteria has been related to the energised state of the membranes which drive proton transport and phosphorylation [1, 2]. Although this absorbance change is one of the most conspicuous phenomena in chloroplasts, there is no general agreement as to its origin. The 515 nm change has been postulated to be confined to grana [3]. Previous works have reported that the two photosystems participate to a similar extent in the generation of the 515 nm signal [4, 5]. Other workers attributed the 515 nm change mainly if not exclusively to Photosystem I [6, 7]. Further information on this problem could be expected from a comparison of chloroplasts with various granum contents which develop Photosystems I and II in different stoichiometry.

Abbreviations: PMS, *N*-methylphenazonium methosulphate; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; $\Delta I/I$, light induced absorbance change.

MATERIALS AND METHODS

Chloroplast isolation. Chloroplasts with high and low granum content were prepared from mesophyll and bundle sheath cells of maize (*Zea mays* l cv. INRA 250). The plants were grown in a greenhouse for 10–14 days. Separation of chloroplasts was performed from the first leaves of the seedlings by using the multistep grinding technique and a medium containing 0.4 M D-sorbitol, 0.05 M Tris · HCl, 0.01 M sodium ascorbate and 0.005 M cysteine · HCl, pH 7.8 [8]. Care was taken to avoid any harsh treatment in order to obtain a large percentage of intact (Class A and Class B) chloroplasts. The chloroplasts were collected by centrifugation at $1200 \times g$ (10 min) and the pellets were resuspended in a medium containing 40 % buffer (0.1 M sucrose, 0.05 M Tris · HCl, pH 7.0) and 60 % glycerol to reduce sedimentation and to improve the optical properties of the sample. The chlorophyll content was adjusted to 20–25 nmol/ml. The granum content of the samples was monitored under the electron microscope.

Measurement of the absorbance change. A 10×10 mm cell containing the chloroplast suspension was inserted into a holder which maintained the temperature of the sample at approximately 3 °C. The cell was illuminated through a horizontal light guide by xenon flashes (General Radio, Stroboslave, 3 μs, energy 0.5 *J*) at a right angle to the measuring beam: Actinic light passed through a Calflex C (Balzers) filter and a Schott RG 630-3 mm filter. Flashes were fired at 4 s intervals (unless otherwise indicated). The measuring light was obtained by means of a Bausch and Lomb 500 lines/mm monochromator (slits 1.0 mm) and after passing the sample was led by a light guide to an EMI 9559 B photomultiplier protected by a Schott BG 18-3 mm filter. The intensity of the measuring beam was around 3 ergs · cm^{-2} · s^{-1} at 515 nm and varied slightly with the wavelength. 50 to 400 signals were measured and collected in a Didac 800 multichannel analyser (further details in ref. 9). During the measurements (4–30 min) no appreciable sedimentation of the samples occured, and the signal did not decrease by more than 2–10 %.

RESULTS AND DISCUSSION

Amplitude and decay of the 515 nm change

In chloroplasts of low granum content the flash-induced 515 nm change was small as compared to that obtained with chloroplasts with high granum content (Figs. 1A and 1C). In the time range of analysis both signals consisted of two components, one with a fast $T_{\frac{1}{2}} \approx 10$ ms and the other with a slower $T_{\frac{1}{2}} \approx 100$ ms decay. The gramicidin-D insensitive part of the absorbance change represented 20 % of the initial amplitude observed with granal chloroplasts and was relatively higher in chloroplasts of low granum content (Figs. 1B and 1D).

The gramicidin-sensitive 515 nm signal measured with chloroplasts of low granum content was 31 ± 7 % of the corresponding signal of chloroplasts with high granum content. As shown in our earlier measurement [10] and, in some recent studies, under the electron microscope, granum thylakoids of mesophyll chloroplasts represent 60 % of the total thylakoid content, while in bundle sheath chloroplasts this value is about 8–12 %. Cross contamination of the samples varied between 5 and 30 %, so the estimated granum content of "high grana" preparations could vary

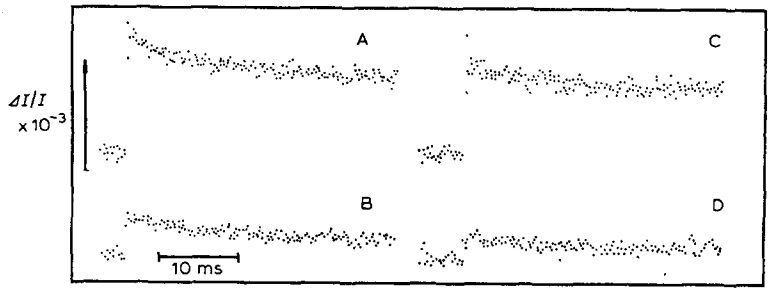

Fig. 1. Oscilloscope display of the absorbance changes at 515 nm for granal and agranal chloroplasts before and after gramicidin treatment. A and B: granal chloroplasts, chlorophyll $(a+b)$: $2.1 \cdot 10^{-5}$ M, chlorophyll a/chlorophyll b: 3.2 C and D: agranal chloroplasts, chlorophyll $(a+b)$: $2.2 \cdot 10^{-5}$ M, chlorophyll a/chlorophyll b: 4.6 Upper part: without gramicidin D, lower part: with 10^{-6} M gramicidin D. Optical path: 1.0 cm. Temperature 3 °C. Number of transients in the average: 100 for A, B and 200 for C, D.

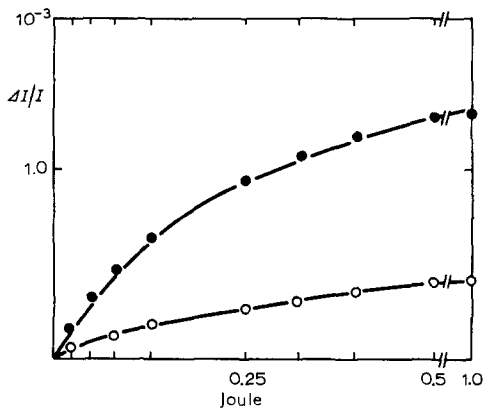

Fig. 2. Amplitude of the gramicidin-sensitive 515 nm signal as a function of the flash energy. ●, granal chloroplasts; ○, agranal chloroplasts. The maximum energy was given by two synchronized flashes from two sides of the cuvette, attenuation was achieved by neutral filters. Measuring conditions as in Fig. 1.

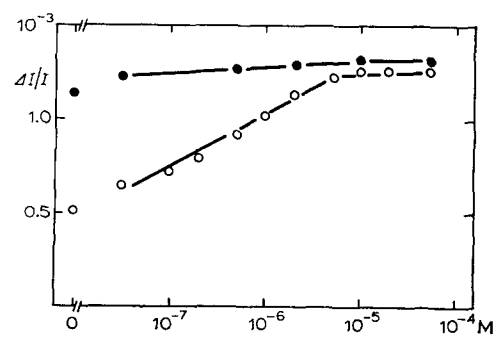

Fig. 3. Amplitude of the gramicidin-sensitive 515 nm signal as a function of the concentration of PMS added to the suspension of granal and agranal chloroplasts. ●, granal chloroplasts, ○, agranal chloroplasts. Measuring conditions as in Fig. 1.

between 57 % (low contamination) and 45 % (high contamination). Corresponding values for preparations of "low granum" content were 12 and 25 %, respectively. In four experiments, with 3000 chloroplasts counted, the granum content of the bundle sheath chloroplast preparations was 28±9 % of that of the mesophyll chloroplasts, thus the amplitude of the gramicidin-sensitive 515 nm change correlated fairly well with the granum content in the samples. Nevertheless, it cannot be excluded that agranal thylakoids exhibit either a small or a fast decaying signal difficult to detect. As in some other photosynthetic reactions [11], the intensity of the flash might not be enough to energise the agranal bundle sheath thylakoids. However, we found that the dependence of the 515 nm change on the energy of the actinic flash was similar with mesophyll and bundle sheath chloroplasts and the ratio of the magnitude of absorption change of the two types of chloroplasts was constant over a large range of flash energy (Fig. 2).

The 515 nm signal of granal and agranal chloroplasts was compared with 4 and 8 sec intervals between the flashes and the results were the same. This suggests either that a complete relaxation of the system occurs (within 4 s) or alternatively that a very long time is required for the relaxation of the factor limiting the 515 nm change of agranal chloroplasts.

The effect of PMS

In attempts to induce (or increase) the 515 nm signal of agranal chloroplasts, the addition of PMS was found to be very efficient. Upon addition of a saturating amount of PMS the signal of agranal chloroplasts preparations increased by a factor of 2–4, while in granal chloroplasts only a marginal enhancement was observed.

PMS effected mainly that part of the signal which could be abolished by gramicidin. As a result, the 515 nm change in agranal chloroplasts became as large as that in mesophyll chloroplasts (Fig. 3). The suggestion that PMS can activate some silent Photosystem I was also supported by the experiments carried out in the presence

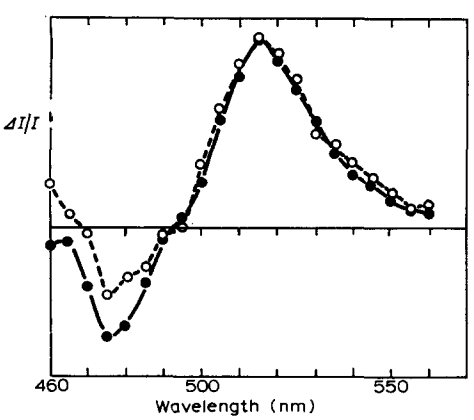

Fig. 4. Wavelength dependence of the gramicidin-sensitive absorbance changes of granal and agranal chloroplasts. For the sake of comparison $\Delta I/I$ was normalized to the same value at 515 nm. ●, granal chloroplasts; ○, agranal chloroplasts. The suspensions were supplemented by 10^{-5} M PMS. Measuring conditions as in Fig. 1.

TABLE I

Peak-heights in the light-dark difference spectra of mesophyll and bundle sheath chloroplasts without and with 10^{-5} M PMS. Chlorophyll contents: $2.0 \cdot 10^{-5}$M for the mesophyll chloroplasts, $2.2 \cdot 10^{-5}$M for the bundle sheath chloroplasts. Chlorophyll *a*/chlorophyll *b*: 3.4 and 4.1, respectively. The values of the absorbance change were corrected for the gramicidin insensitive part (positive at both wavelengths)

	PMS addition	$\frac{\Delta I}{I} \times 10^3$ per 100 flash at 515 nm	$\frac{\Delta I_{515nm}}{\Delta I_{475nm}}$
Mesophyll chloroplast	−	0.96	1.7
(high granum content)	+	1.05	1.8
Bundle sheath chloroplast	−	0.24	1.6*
(low granum content)	+	1.01	3.2

* Uncertain value, because the 475 nm peak was very much influenced by the gramicidin insensitive signal and the noise. For this value 400 flashes were collected, for the others only 50.

of DCMU (Table II). In both mesophyll and bundle sheath chloroplasts the suppression of Photosystem II activity (by DCMU and DCMU plus NH_2OH) resulted in a 30–40 % decrease of the 515 nm signal provided PMS was absent. Upon addition of PMS to the samples inhibited by DCMU the amplitude of the 515 nm signal was largely increased. As a result, the 515 nm absorbance change rose near to the level which was observed in chloroplasts treated with PMS only.

The signal generated after PMS treatment was a characteristic 515 nm absorbance change as shown by the light-minus-dark difference spectrum of the gramicidin-sensitive component. Fig. 4 shows the close similarity between the difference spectra of mesophyll and bundle sheath chloroplasts, except for the negative band centered at 475 nm.

Such a difference was not detectable prior to PMS treatment, when the peak ratio $(\Delta I/I)_{515\,nm}/(\Delta I/I)_{475\,nm}$ was the same for mesophyll and bundle sheath chloroplasts (Table I). The low $\Delta I/I_{475\,nm}$ value in the absorbance change induced in the

TABLE II

The effect of PMS and DCMU on the amplitude of the gramicidin-sensitive 515 nm absorbance change of granal and agranal chloroplasts. Measuring conditions as in Fig. 1.

	Granal chloroplasts	Agranal chloroplasts
Control $\Delta I/I \times 10^3$ per 100 flash	1.24	0.36
%	100	100
+10 μM PMS*	105	252
+10 μM DCMU**	74	61***
+10 μM DCMU plus 10 μM PMS	92	240

* Addition of ascorbic acid had no appreciable effect, probably because the chloroplasts were saturated with the ascorbic acid of the isolation medium.

** Supplementing the DCMU by 1 mM NH_2OH was ineffective.

*** 400 flashes collected.

presence of PMS indicated that membrane components activated by PMS were probably Photosystem I entities which are poor in chlorophyll *b*, and responsible for the negative signal at 475 nm.

The decay kinetics of the PMS stimulated gramicidin-sensitive signals appeared to be similar to those of the controls ($T_{\frac{1}{2}} \approx 10$ ms). (Detailed analyses could not be performed, since the PMS induced increment in granal chloroplasts and the control signal of agranal chloroplasts were too small for comparative kinetic studies under the experimental conditions applied).

In mesophyll chloroplasts PMS induced only a marginal enhancement suggesting a low effect on "native" grana in freshly isolated chloroplasts. Apparently the stroma thylakoids which make up 40 % of the total thylakoid content and are supposed to contain Photosystem I only, were not activated or their activity could not be enhanced to an appreciable extent. After longer storage, however, mesophyll chloroplasts also responded to PMS-treatment, albeit to a lesser extent. This phenomenon can be essentially the same as observed with aged spinach chloroplasts [12]. As to the different effect of PMS on mesophyll and bundle sheath chloroplasts, it should be borne in mind that in bundle sheath chloroplasts there is a 3-fold higher Photosystem I/Photosystem II ratio than in granal chloroplasts [13, 14]. Under such conditions only a part of Photosystem I can be coupled to Photosystem II by the electron transport chain and the rest can recycle only at the expense of some external electron donor. These unconnected Photosystem I entities might be the target of PMS, which substitutes the electron transport chain [15] and, as a result, "idling" Photosystem I entities become activated.

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